Non-T, Non-B Lymphomas Are Rare in Childhood and Associated With Cutaneous Tumor

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Tumor cells from a total of 116 children with non-Hodgkin's lymphoma were studied for their pattern of reactivity with a battery of cell markers, including their capacity for spontaneous formation of sheep erythrocyte rosettes (E-rosettes), demonstration of surface immunoglobulins (Slg), and positivity with antisera against T-cell antigens, the common acute-lymphoblastic leukemia-associated antigen (CALLa), and Ia-like antigens. Fifty-eight children (50%) had T-cell lymphomas, including all those with mediastinal tumors. Fifty children (43%) had B-cell lymphomas, including 44 of the 45 with abdominal primaries. Eight children (7%) had non-T, non-B tumors, 4 of whom presented at a young age with cutaneous lymphoblastic tumors. These results demonstrate that the great majority of children with NHL, not leukemic at diagnosis, have tumors clearly committed to either T- or B-cell differentiation pathways and only rarely exhibit the common ALL phenotype (CALLa+, Ia+, E-, T-, Slg-), contrasting with the distribution of childhood lymphoblastic leukemias. The unusual association of these non-T, non-B cases with skin involvement has not previously been reported, raising speculation regarding patterns of lymphocyte traffic and origins of childhood lymphomas and leukemias.

PREVIOUS STUDIES of the tumor cell markers of malignant lymphomas of childhood have disclosed evidence for approximately equal frequencies of cases committed to either T- or B-cell pathways of differentiation, with only rare cases classified as unmarked, so-called "null" or "U" type. Furthermore, there is a high degree of predictability of the immunologic phenotype of the lymphoma cells based on the morphological subclassification and a close correlation with the clinical presentation of disease. T-cell lymphomas in children typically have lymphoblastic morphology and either mediastinal and/or nodal involvement, whereas B-cell lymphomas commonly arise in the abdomen and are composed of small or large noncleaved cells of so-called "undifferentiated" or Burkitt-like histology.

Other investigators have blurred the conventional clinical and anatomic distinctions between lymphomas and leukemias in children by reporting a high proportion of cases of "null" lymphoblastic disease, with few exceptions basing these observations on studies of either marrow or peripheral blood from frankly leukemic cases. This has led many to consider childhood lymphomas and leukemias as differing phases of the same disorder, despite the fact that numerous series of marker studies in ALL attest to the preponderance of the non-T, non-B phenotype. The leukemic cells from the majority of cases of ALL tested in childhood lack either detectable T antigens or surface or cytoplasmic immunoglobulins, instead expressing a common antigen, CALLa and Ia-antigens.

The present series of cell marker studies from children with lymphoma not in a leukemic phase at diagnosis has been combined from two pediatric cancer centers. Our combined series is presented to emphasize the rarity of the common ALL phenotype in NHL and to call attention to the unusual localization of tumor in the skin in these cases. These observations of the tumor cell characteristics are relevant to promotion of a clearer definition of the biologic distinctions between lymphomas and leukemias in children and raise speculation regarding the skin as an immunologic microenvironment.

MATERIALS AND METHODS

Patients

The 116 children in this combined series were seen either at St. Jude Children's Research Hospital from November 1977 to November 1980 or at Institut Gustave-Roussy from November 1976 to November 1980. The series includes 39 cases reported previously. The cases studied represent roughly one-half (48.7%) of all children with NHL admitted to the two institutions during the same time period. The availability of fresh tumor cells for study in children with NHL depends on the site(s) and initial extension of disease as well as on referral practices. Fresh tumor cells were unavailable for study from the other half of all NHL cases, usually because they had localized disease and were biopsied elsewhere. Consequently, the present series is weighted toward cases with initial stage III or IV disease, according to a staging system described elsewhere. All children were aged less than 19 yr at diagnosis and had typical lymphomatous tumor presentations. The diagnosis of lymphoma was established by standard morphological and histochemical criteria. All patients underwent one or more initial bone marrow aspirations and had none or less than 25% lymphoma cells in otherwise normal marrows at the time of diagnosis.

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Tumor Specimens

Neoplastic nodes or tumors were gently teased and minced to form single cell suspensions and passed through wire mesh. Tumor cells from malignant pleural or ascitic effusions, from cerebrospinal fluid, or from solid tumor suspensions were separated, when necessary, from red cells by Ficoll-Hypaque gradient centrifugation. All cases from Villejuif represent extramedullary tumor sampled at the time of diagnosis, whereas the Memphis series includes 9 samples of marrow from patients tested at relapse. We have observed constancy in the marker phenotype of patients' cells tested at diagnosis and again at relapse and uniformity in marker expression from multiple involved sites sampled simultaneously in an individual. Tumor cells were readily distinguished from normal cells in cell suspensions by their morphology on Wright-Giemsa-stained cytocentrifuged smears of cell preparations that were routinely 80%-100% neoplastic.

Surface Immunoglobulins

Cells bearing surface immunoglobulins were detected by direct immunofluorescence using goat anti-human immunoglobulins (Cappel Lab, Inc., Downingtown, Pa.) following techniques previously described. Where possible, tumor cell populations were tested for monoclonal SIg with heavy and light chain specific reagents.

E-Rosette Formation

Rosette formation was assayed using sheep red blood cells pretreated with 2-amino-ethylisothiouronium bromide hydrobromide (AET). Morphological identification and confirmation of the neoplastic nature of the E-rosetting cells was performed on Wright's-stained smears.

Preparation and Specificity of Heteroantisera

The preparation of the rabbit anti-T antisera used in Villejuif and the specificity for T-cell antigens has been extensively described elsewhere. Briefly, 11 anti-human T-cell antisera were raised in rabbits by injection of various normal or neoplastic T cells and were made specific for cells of the T lineage by appropriate absorptions of AB + red blood cells, pooled human platelets, and B-lymphoid cell lines. The anti-T-cell antisera used in Memphis were prepared against fetal thymocytes or blast cells from T-ALL and made specific for T cells as described. The Memphis anti-T antisera recognized 80% of human peripheral blood lymphocytes, all T-cell lines tested, blast cell specimens from all E-positive ALL cases as well as approximately 20%-30% of E-negative common ALL-positive leukemic blast cell specimens. Heteroantisera recognizing cALLa were raised in rabbits, as described by Greaves. The cALLa-antisera recognize lymphoblasts from 60% of cases of ALL in children and do not react with thymocytes or nonlymphocytic leukemic cells or normal peripheral blood cells. The immunofluorescent patterns of reactivity and the SDS-PAGE analysis characteristic of the cALLa antisera used in Memphis have been reported elsewhere and the antisera proven to recognize the gp 100 cALLa antigen. The cALLa antisera prepared in Villejuif reacts with a similar surface component with a mol wt of approximately 110,000 on SDS-PAGE analysis (R. Frade, manuscript in preparation). The heteroantisera recognizing Ia-like antigens used in Memphis were prepared as described elsewhere and reacted with normal and neoplastic B cells and monocytes and were unreactive with thymocytes and peripheral blood T cells. At Villejuif, a series of different reagents for detection of Ia-like antigens was used over the time period studied, including first a panel of 30 alloantisera recognizing 7 different HLA-DR allosepecificities, later a heteroantisera prepared as described by Schlossman et al., and most recently a monoclonal anti-Ia antibody.

Phenotyping of Tumor Cells With Antiseras

In Memphis, tumor cells were tested in an indirect immunofluorescence assay as follows: washed viable cells were incubated with test antisera at 4°C in the presence of sodium azide and antibody binding made visible with a second reagent, fluorescein-conjugated polyvalent goat anti-rabbit immunoglobulin (Behring Diagnostic). Samples were considered positive when more than 40% of the cells showed positive surface fluorescence. In Villejuif, cells were tested using a complement-dependent microcytotoxicity test and the viability was assessed with trypan blue exclusion. Samples were considered positive when more than 80% of the cells were lysed by the antisera plus complement; negative when less than 20% of the cells were killed.

Assay for Terminal Deoxynucleotidyl Transferase (TdT)

Assay for TdT activity by the biochemical technique was performed with reagents and methodology as described previously.

RESULTS

Distribution of Childhood NHL Cases, According to Primary Site and Cell Marker Analysis

Table 1 shows the overall distribution of NHL cases from the combined center's experience, according to primary site and T- and B-cell marker lineage. There is a high degree of predictability of the immunologic marker from the site of origin of the primary tumor. All of the 50 children presenting with mediastinal NHL had tumors of T-cell origin, as evidenced by detectable T antigens, and all but one of the children with abdominal (46) or epidural tumor (3) had B-cell disease, based on demonstrable tumor cell surface immunoglobulins and Ia antigens. Seven of 10 patients with primary nodal disease had T-cell lymphomas.

One-hundred-eight of the 116 cases tested (93%) were clearly either T- or B-cell in origin, whereas only
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8 had neither detectable T antigens nor SIg. These 8 cases are described in more detail below.

**Characteristics of the Non-T, Non-B Cell NHL Cases**

Eight of the cases tested fell into a marker pattern that was not definable as T or B, and 4 of these 8 had the remarkable association of initial skin involvement along with the cell marker phenotype characteristic of the common form of childhood lymphoblastic leukemia, despite not being in a leukemic phase of their disease at diagnosis.

Table 2 outlines the age at diagnosis, sex, initial sites of involvement, and clinical stage of these four children with skin primaries whose tumor cells exhibited only the Ia-like antigens and the cALLa antigen. In three of the four cases that could be tested, increased quantities of the enzyme TdT were found. Three of the four children clearly had localized disease, stage I or II, whereas the one patient with generalized skin involvement also had widespread visceral involvement and initial partial marrow infiltration. These four cases were notably younger than the usual mean age of 7–9 yr expected for children with NHL. Three of the four of the non-T, non-B skin cases had lymphoblastic nonconvoluted morphology by light microscopic examination of the tissue sections and demonstrated diffuse infiltration of lower layers of the skin, sparing the upper dermis, papillary dermis, and epidermis (Fig. 1).

In the other skin case, the lymphoblasts exhibited definite convolutions in tissue sections.

The other four cases of non-T, non-B NHL were somewhat more heterogenous in their clinical presentation, though the cell markers from three of the four showed Ia-like antigens plus cALLa. Similarly, these 3 cases showed lymphoblastic nonconvoluted morphology, and two of the three initially were stage IV with partial marrow involvement in association with tumors in the head and neck region. The fourth non-T, non-B case was positive only for Ia-like antigens, being an abdominal tumor case with Burkitt-like histology and otherwise unaccountable absence of SIg. We cannot exclude the possibility that these cases may represent pre-B-cells, as we did not systematically search for cytoplasmic immunoglobulins.

The expression of the cALLa antigen on the tumor cells in NHL was therefore rather limited in our series, being present in seven of these eight cases of non-T, non-B lymphomas. In addition, we observed the cALLa in 12 of the 50 cases of mediastinal lymphomas tested, generally exhibiting somewhat fainter indirect immunofluorescence than usually observed in ALL cases. Less often, we observed cALLa positivity in some cases of B-cell (SIg+) tumors.

**DISCUSSION**

These results demonstrate that most lymphomas in children (>90%) arise either in T- or B-cells in a frequency and distribution similar to that reported by other authors. Dura et al. reported 52% B-cell, 36.7% T-cell, and 6.9% undefined "U" type of lymphomas in a morphological and immunocytochemical study of 211 childhood cases, and Crist et al. observed similar percentages of 42% B, 52% T, and 6% non-T, non-B in their collected series of 130 cases of childhood NHL. We found 43% B-cell, 50% T-cell, and 7% non-T, non-B, remarkably parallel results obtained with different reagents and techniques. Even though our cell marker results were obtained largely from study of children with advanced stage disease, we believe the results to be representative of the cellular origins of localized NHL as well, given the very high degree of predictability of the immunologic phenotype of lymphoma cells by conventional morphology and recognizing that the morphological distribution of cases of localized lymphomas does not substantially differ from the distribution of cases of advanced disease.

Only in a small minority of cases (7%) did we observe the non-T, non-B cell phenotype, all the more remarkable since 4 of the 7 cases with tumor cells positive only for cALLa and Ia-like antigens were localized in the skin in very young children. Skin involvement in NHL is unusual in children at diagnosis, the 5 cases reported here being the only 5 we observed in all children presenting with NHL during the entire period under study in both centers, i.e., 5 of 234 (2%). Except for one case that clearly showed cellular convolutions, the non-T, non-B cALLa-positive lymphomas we observed all were composed of cells without distinct morphological-cytologic characteristics, i.e., lymphoblastic nonconvoluted.

Epidermotropism is the characteristic finding of mycosis fungoides, Sézary syndrome, and related disorders. lymphomas composed of mature T cells. Our finding of preferential cutaneous localization by
non-T, non-B, cALLα-positive lymphoma cells has not previously been reported, and the biologic significance of this association can only be the source of speculation at present. Streilein has advanced the notion that there exists a special inductive interaction between lymphocytes and the skin and a traffic of lymphocytes between lymphoid organs and epithelium.24 If one adopts the view that lymphomas represent clonal expansions of the normal anatomic and functional counterparts of the immune system, then our rare finding of cALLα-
positive, Ia-positive lymphomas localized to the skin in very young children may suggest the existence of a normal counterpart lymphocyte whose identity and function has not yet been recognized.

It is not altogether unexpected that we observed the cALL antigen on the small minority of our cases of non-T, non-B lymphomas, but it deserves emphasis that we also observed cALLa-positivity in a small percentage of T-cell and B-cell lymphomas, confirming the emerging viewpoint that the cALL antigen is neither restricted to a particular line of lymphoid differentiation nor confined to an early stem cell class. In a survey of 545 adults and children with various hematopoietic malignancies, Greaves and coworkers found the cALL antigen on the majority of cases of non-T, non-B ALL, 10% of T-ALL, 45% of PH1-positive blast crises of chronic myelocytic leukemias, and in some lymphomas tested, though with very few exceptions the lymphoma cases tested had bone marrow involvement and were actually bona fide common ALL cases. The finding of cALLa reactivity on neoplastic cells related to common and late thymocytes has also been observed in a series of 21 cases of childhood lymphoblastic lymphomas studied with the OKT series of monoclonal antibodies. Nadler and Ritz have reported that the monoclonal J5 antibody to cALLa developed by Ritz reacts with cases of Burkitt's lymphoma and nodular and diffuse types of poorly differentiated lymphocytic lymphomas as well. The likely possibility that the method of detection of cALLa, either by heteroantisera or by monoclonal antibodies, may account for some of the diversity in the reported findings should be considered, particularly in view of the evidence presented by Pesando et al. indicative of heterogeneity of the specificities corresponding to the accepted definition of cALLa. The monoclonal J5 antibody recognizes a specificity that is definitely shared by T, B and non-T, non-B cells. Further development of monoclonal antibodies recognizing additional epitopes of cALLa different from the one recognized by the J5 may demonstrate a variety of surface molecules, leaving an opening for the possibility of a unique molecular species of cALLa characteristic of lymphoid cells within a particular pathway of differentiation. Taken together with the results obtained with heteroantisera, such an approach would help to answer the interesting question of whether or not the non-T, non-B lymphomatous cells we have seen in our patients are exactly the same as or different from the leukemic cells of the common form of childhood ALL.

Overall, the marker data presented have provided evidence for significant differences in the state of lymphoid cell differentiation characteristic of the lymphomas and the leukemias of childhood. Most lymphomas would be of extramedullary origin, either T or B, whereas the majority of childhood leukemias are non-T, non-B, derived from bone marrow cells. Even within a particular lineage, however, there is evidence for phenotypic differences between malignant T cells from young patients with ALL and NHL. We can readily agree with the viewpoint that it is scientifically unwarranted to make an arbitrary decision between lymphomas and leukemias based solely on the degree of marrow infiltration, being aware that a leukemic phase is often associated with lymphoma evolution in children. But we do not favor the tendency to lump all leukemias and lymphomas of childhood together, as this tendency may mask significant pathologic and biologic features of the neoplastic disease process. Future studies incorporating additional markers of lymphocyte differentiation, such as the studies of gene regulation of Waldmann et al. should advance our understanding of the biology of lymphoid neoplasia and will surely reduce still further the already low proportion of unclassifiable cases of childhood lymphomas encountered.

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